115. (new) The vaccine composition of Claim 8, wherein said peptide or protein is encoded by the nucleotide sequence selected from the group consisting of: SEQ ID NOS: 1, 3, 5, 6, 8, 11, 13, 15, 17, 18, 19, 20, 21, 22, and 23.

REMARKS

The Specification has been amended to correctly identify trademarks. The claims have been amended to correct minor informalities and to more clearly claim the invention. Claims 1, 2, 6-10, 12, 32, 37-41, 43, 94, 95, 108, 109 and 114 and 115 are presented for further examination. No new matter has been added herewith.

The changes made to the specification by the current amendment, including **[deletions]** and additions, are shown on an attached sheet entitled **VERSION WITH MARKINGS TO SHOW CHANGES MADE**, which follows the signature page of this Amendment.

Rejections under 35 U.S.C. §112, second paragraph -

The Examiner believes that Claims 1, 6-10, 12, 32, 37-41, 43, 94, 95, 108 and 109 are indefinite for the following reasons:

Claims 1 and 6 recite a "related microorganism", thus, Claim 1 and 6 have been amended to specify "wherein said related microorganism is an isolate or subspecies of *L. intracellularis* or other species of the genus *Lawsonia*".

Claim 40 is believed indefinite as to "derivative thereof", however, claim 40 has been amended to read "wherein said derivative is still immunogenic".

Claim 41 is believed indefinite as to "the protein is GroEL" however, Applicants believe the Examiner is referring to a different claim.

Claim 9 and 40 are believed vague as to "refolding/heat shock" protein and "S-adenosylmethionine: tRNA ribosyltransferase-isomerase" protein. However, Applicants submit that the name of the protein is not indefinite because when proteins have more than one enzymatic function, they are commonly referred to by reciting both functions with a "\" or a ":" between them.

Claims 37, 38 and 39 recite "peptide", "protein", and "polypeptide". These claims have been amended to recite "peptide, protein" which is believed definite as one of skill in the art would know that a peptide is a small protein or part of a protein.

Claims 10 and 94 are believed indefinite as to the term "immunologically". The claims have been amended to recite "immunogenically".

Claim 109 lacks antecedent basis for the recitation "The method of claim 12" since claim 12 is not a method claim. Claim 109 has been amended to recite "The method of Claim 41".

Claim 94 and 95 are believed indefinite as to the recitation "under hybridization conditions.", because the temperature was not recited. However, the claims have been amended to read " at a temperature of 42°C". Support for this amendment can be found on page 10, line 7.

Thus, Applicants submit that in view of the above amendments and arguments, the claims are definite.

Rejections under 35 U.S.C. §112, first paragraph -

The Examiner believes that Claims 41 and 43 are not enabled because the claims recite a protein having "at least 40%" similarity to a GroEL protein" and this is not sufficiently explained. The Examiner believes that the addition of specific algorithms is necessary. However, Applicants disagree and submit that the methods for determination of % similarity are found in many commonly used computer programs, such as Blast, etc. and that one of skill in the art would use these programs to determine whether a protein is at least 40% similar. In addition, because GroEL proteins are known to be similar from one species to the next as are many evolutionarily conserved proteins, it is likely that homologs would be even more similar than 40%.

The Examiner believes that Claims 38-43 are not enabled because the instant claims are drawn to a method for vaccinating an animal against *L. intracellularis* or a related microorganism by administering a "derivative" of a "peptide, polypeptide, or protein" from the organism. The Examiner does not believe there is enablement for a "derivative". However, the amended claims recite that the derivative must be "immunogenically reactive". Applicants believe that one of skill in the art would be able to identify an "immunologically reactive" derivative by identifying the antigenic region experimentally or using a computer program.

Rejection 1 under 35 U.S.C. §102(b) -

The Examiner believes that Claims 1, 2, 6, 7, 32, 37 and 38 are anticipated by Knittel et al (US 5,714,375). The Examiner believes that Knittel et al. disclose vaccines comprising an attenuated or avirulent immunogenic strain or antigen of Ileal symbiont intracellularis (*Lawsonia*

intracellularis). However, Knittel, et al is drawn to a method for growing *L. intracellularis* in a way that is inducive to the production of an attenuated bacteria which can be used for a vaccine, and they refer to this vaccine as an "antigen". Thus, the "antigen" taught by Knittel et al. is the whole attenuated bacteria. Knittel does not teach or suggest an immunogenic component or subunit-type vaccine. Amended Claim 1 recites an immunogenic component of *L. intracellularis*.

Rejection 2 under 35 U.S.C. §102(b) -

The Examiner believes that Claims 1, 2, 6-9 and 94 are anticipated by Labigne et al. (WO 94/26901). However, Labigne, et al. disclose a recombinant immunogenic composition of *Helicobacter felis* or *H. pylori*, which may be related to *Lawsonia intracellularis*, but is not the same genus. The amended claims recite: "wherein said related microorganism is an isolate or subspecies of *L. intracellularis* or other species of the genus *Lawsonia*". Thus, Labigne does not anticipate claims 1, 2, 6-9 and 94.

Rejection 1 under 35 U.S.C. §103(a) -

The Examiner believes that Claims 32, 37-41, 43, 94 and 95 are obvious in view of Labigne et al (WO 94/26901). Specifically, the Examiner believes that although Labigne does not expressly teach a method of vaccinating with their recombinant composition, it does suggest immunizing for veterinary purposes. However, *prima facie* obviousness requires a recitation of all of the claimed elements. Labigne does not teach all of the claim elements because Labigne et al does not teach the specific microorganism. Specifically, because amended claims 32, 37-41, 43, 94, and 95 specify "wherein said related microorganism is an isolate or subspecies of *L. intracellularis* or other species of the genus *Lawsonia*", Labigne does not render the method obvious because Labigne teaches a method for vaccination against a completely different microorganism which is not a species of the genus *Lawsonia* (It is genus Helicobacter).

Rejection 2 under 35 U.S.C. §103(a) -

The Examiner believes that Claims 1, 2, 6-8, 32 and 37-39 are obvious in view of Joens et al. (US 5,610,059). The Examiner believes that although Joens does not expressly teach a method of vaccinating with their recombinant composition, it does suggest immunizing for veterinary purposes. However, *prima facie* obviousness requires a recitation of all of the claimed elements. Joens does not teach all of the claim elements because Joens et al does not teach the

specific microorganism: a method of vaccinating with *L. intracellularis*. Joens et al. simply identifies a PPE causing organism and suggest ways of producing and identifying immunogenic proteins and whole organism vaccines, methods which were known in the art. Thus, Joens et al. does not teach a method of vaccinating with recombinant or subunit from *L. intracellularis* or species of Lawsonia, nor any proteins which are specifically immunogenic and Joens et al does not render the presently claimed invention obvious.

Conclusion

Should there be any questions regarding the above-identified patent application, the Examiner is respectfully requested to contact the undersigned at the telephone number appearing below.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: 2/22/02

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION

On page 21, third paragraph (EXAMPLE 4) starting on line 18 and ending on line 24, please cancel the paragraph and insert:

--A lambda ZAP II™ *L. intracellularis* genomic library was plated on a lawn of *Escherichia coli* XLI-Blue™ (23) cells at a density of 2,000 plaque-forming units (pfu) per 150 mm L-broth agar plate. The library was screened with a rabbit anti-*L. intracellularis* sera using the method described in the Protoblot™ Technical Manual (Promega, WI, USA). Filters were blocked in a buffer containing 10mM Tris HCl, pH8.0, 150mM NaCl, 0.05% Tween 20™, 1% w/w gelatin. Positive plaques identified in a primary screen were picked, replated at a lower density and rescreened until individual positive plaques were identified.--.

On page 21, fourth paragraph (EXAMPLE 5), starting on line 29 and ending on page 22, line 4, please replace the paragraph with the following:

--Phagemid DNA from positive λZAP II™ phage clones was isolated by excision *in vivo* of the pBluescript™ phagemid under the conditions recommended by Stratagene (CA, USA). Plasmid DNA was either extracted by the method of Birnboim and Doly and the cDNA inserts sequenced by the chain termination method (21), or by the PEG-precipitation method and cycle-sequenced by the dye-terminator method, as recommended by the manufacturer (Applied Biosystems).--.

On page 22, second paragraph (EXAMPLE 6), starting on line 9 and ending on line 14, please replace the paragraph with the following:

--Antisera to *L. intracellularis* bacteria were raised in rabbits and pigs. Rabbits were injected intramuscularly with a preparation of Percoll™ gradient-purified *L. intracellularis* bacteria mixed with a double-emulsion made by processing with oil adjuvant (Freund's incomplete adjuvant, CSL Limited, Melbourne, Australia), and then with Tween 80™ enhancer. Two 3 ml injections, containing 9 mg protein, were given four weeks apart. Blood samples were collected from the marginal ear vein prior to immunisation and two weeks following the second injection.--.

On page 22, third paragraph (EXAMPLE 6), starting on line 16 and ending on line 21, please replace the paragraph with the following:

--A 6-week old pig (395) was hyperimmunised by intramuscular injection of Percoll™ gradient purified *L. intracellularis* bacteria prepared with Freund's incomplete adjuvant as for the rabbit. Three injections of the prepared antigen were administered four weeks apart, and blood was collected from the jugular vein two weeks following the final injection. Diluted pig sera (1 ml, 1 in 200) were pre-adsorbed with 100 µl *E. coli* DH5α (24) lysate for 1 h at room temperature with gentle mixing. The lysate was prepared by freeze-thawing a suspension of *E. coli* in PBS.--.

On page 23, first paragraph (EXAMPLE 8), starting on line 4 and ending on line 11, please replace the paragraph with the following:

--Proteins were electrophoretically transferred to Immobilon-P™ (Millipore Corporation, MA, USA) membranes in a Trans-Blot Cell™ (BioRad, CA, USA) at 100 V for 1 h in a buffer containing CAPS (3-[Cyclohexylamino]-1-propanesulfonic acid, pH 11, Sigma, MI, USA) and 10% v/v methanol. The membranes were then blocked with 5% w/v Blotto™ (Diploma skim milk powder, Melbourne, Australia) in PBS for 30 min at room temperature with gentle rocking. The filters were then transferred to antisera diluted in 5% w/v Blotto™, PBS. Pre-adsorbed pig antisera was diluted 1 in 200. The filters were incubated in pig antisera for 1 h followed by washing three times in PBST.--.

On page 23, first paragraph (EXAMPLE 8), starting on line 13 and ending on line 17, please replace the paragraph with the following:

--HRP conjugated anti-swine immunoglobulins (DAKO, CA, USA) were applied at a dilution of 1:2000. Enhanced Chemiluminescence™ (ECL, Amersham, IL, USA) was used to discriminate *L. intracellularis* proteins. Prior to ECL™ detection, blots were washed three times for 7 minutes each. The filters were exposed to autoradiographic film (Agfa, NJ, USA) for less than 1 minute before developing.--.

On page 24, second paragraph (EXAMPLE 11), starting on line 23 and ending on line 27, please replace the paragraph with the following:

--The percoll™ gradient purified bacterial *L. intracellularis* pellet was resuspended in 1 ml of 1% formalin in saline and incubated overnight at 4°C. The percoll™ gradient-purified *L. intracellularis* bacteria was then mixed into a double-emulsion made by processing with oil

adjuvant (Freund's incomplete adjuvant, Commonwealth Serum Laboratories, Melbourne, Australia), and then with Tween 80™ enhancer.--.

On page 29, first paragraph (EXAMPLE 18), starting on line 20 and ending on line 27, please replace the paragraph with the following:

--*L. intracellularis* genomic DNA was purified as described in Example 3. The DNA was partially digested with the restriction endonuclease *Sau*3A (Promega) and ligated into Lambda ZAP II Express™ (Stratagene). The lambda library was plated on a lawn of *E. coli* XLI-Blue™ cells at a density of 10,000 pfu per 150 Mm L-broth agar plate. The library was screened, as described in Example 4, with sera from Y12. The pig Y12 was immunised with formalin killed *L. intracellularis*, as described in Example 11 & 12. Vaccinated pigs produced antibodies to a range of *L. intracellularis* proteins, as described in Example 14. A number of phage clones expressing *L. intracellularis* proteins were identified.--.

On page 30, first paragraph (EXAMPLE 19), starting on line 5 and ending on line 11, please replace the paragraph with the following:

--Phagemid DNA from positive λZAP II Express™ phage clones was isolated by *in vivo* excision, by the conditions recommended by the manufacturer (Stratagene). Plasmid DNA, for restriction analysis was extracted by alkaline-lysis, as described by Sambrook et al (12), and for automated sequencing using the High Pure Plasmid Kit™, as recommended by the manufacturer (Boehringer Mannheim). DNA sequencing of inserts was performed by the Dye-terminator method of automated sequencing (ABI Biosystems). The sequences identified are set out in SEQ ID NOS: 5-23 (see Example 20).--.

IN THE CLAIMS

1. **(Twice Amended)** A vaccine composition for administration to an animal, comprising:

[a non-pathogenic form of] an immunogenic component of *L. intracellularis* or related microorganism, wherein said related microorganism is an isolate or subspecies of *L. intracellularis* or other species of the genus *Lawsonia* **[or an immunogenic component thereof]**; and

a pharmaceutically acceptable carrier.

6. **(Twice Amended)** **[A]**The vaccine composition according to Claim 1, wherein said immunogenic component comprises **[a]**at least one macromolecule selected from the group consisting of a **[polypeptide]**peptide, a protein, a carbohydrate, a lipid and a nucleic acid from *L. intracellularis* or related microorganism, said macromolecule being present in an amount effective to induce a protective immune response against *L. intracellularis* or related microorganism.

7. **(Twice Amended)** A vaccine composition according to Claim 6, further comprising a **[polypeptide]** further peptide or protein from *L. intracellularis* or related microorganism.

8. **(Twice Amended)** **[A]**The vaccine composition according to Claim 7, wherein the **[polypeptide]**peptide or protein, is a recombinant **[polypeptide]** peptide or protein.

9. **(Twice Amended)** **[A]**The vaccine composition according to Claim 7, further comprising a compound selected from the group consisting of: a refolding**[/]**and heatshock protein, a flagellar basal body rod protein, an S-adenosylmethionine:tRNA ribosyltransferase-isomerase, an autolysin, an enoyl-(acyl-carrier-protein) reductase **[and]**, a glucarate transporter, and a derivative of any of the above, wherein said derivative is still immunogenic.

10. **(Amended Four Times)** A vaccine composition for administration to an animal comprising an **[immunologically]**immunogenically effective amount of a polypeptide that comprises the amino acid sequence of SEQ ID NO:2 and a pharmaceutically acceptable carrier.

32. **(Amended)** A method for vaccinating an animal against infection by *L. intracellularis* or related microorganism, wherein said related microorganism is an isolate or subspecies of *L. intracellularis* or other species of the genus *Lawsonia* or treating an animal infected by *L. intracellularis*, said method comprising the step of:

administering to said animal an effective amount of **[a non-pathogenic form]** an immunogenic component of *L. intracellularis* or related microorganism, wherein said related microorganism is an isolate or subspecies of *L. intracellularis* or other species of the genus *Lawsonia* **[or an immunogenic component thereof]** for a time and under conditions sufficient to induce a protective immune response against *L. intracellularis* or said related microorganism.

37. **(Twice Amended)** A method according to Claim 32 wherein said immunogenic component comprises at least one of a peptide, **[polypeptide,]** protein, carbohydrate, lipid or nucleic acid molecule or a combination thereof from *L. intracellularis* or related microorganism in an amount effective to induce a protective immune response against *L. intracellularis* or **[a]**said related microorganism.

38. **(Amended)** The method according to Claim 37 wherein said immunogenic component comprises a peptide, **[polypeptide,]** protein or a derivative thereof from *L. intracellularis*, wherein said derivative is still immunogenic.

39. **(Amended)** The method according to claim 38 **[in which]**wherein the peptide **[, polypeptide,]** or protein is in recombinant form.

40. **(Twice amended)** A method according to Claim 32, wherein the immunogenic component is selected from the group consisting of: a **[refolding/heatshock]** refolding and heatshock protein, a flagellar basal body rod protein, an S-adenosylmethionine**[:]**, tRNA ribosyltransferase-isomerase, an autolysin, an enoyl-(acyl-carrier-protein) reductase, **[or]** a glucarate transporter, **[or]**and a derivative **[thereof]**of any of the proteins, wherein said derivative is still immunogenic.

41. **(Amended Three Times)** A method of vaccinating an animal against infection by *L. intracellularis* or related microorganisms or treating an animal infected by *L. intracellularis* said method comprising the step of: administering to said animal an **[immunologically]**immunogenically effective amount of a polypeptide that comprises the amino acid sequence of SEQ ID NO:2 or is at least 40% similar to SEQ ID NO:2, for a time and under conditions sufficient to induce a protective immune response against *L. intracellularis* or a related microorganism, wherein said related microorganism is an isolate or subspecies of *L. intracellularis* or other species of the genus *Lawsonia*.

94. **(Twice Amended)** A vaccine composition for administration to an animal comprising an **[immunologically]**immunogenically effective amount of a polypeptide that is immunologically cross reactive with a polypeptide comprising the sequence of SEQ ID NO: 2 and comprises an amino acid sequence encoded by nucleic acid that hybridizes to the complement of a nucleotide comprising the sequence of SEQ ID NO: 1 under hybridization

conditions comprising at least about 16% (v/v) formamide to at least about 30% (v/v) formamide and at least about 0.5M salt to at least about 0.9M salt at a temperature of 42°C.

95. **(Twice Amended)** A method of vaccinating an animal against infection by *L. intracellularis* or related microorganism or treating an animal infected by *L. intracellularis* said method comprising the step of: administering to said animal an immunologically effective amount of a polypeptide that is immunologically cross reactive with a polypeptide comprising the sequence of **[a polypeptide comprising the sequence of]** SEQ ID NO:2 **[and comprises]** or an amino acid sequence encoded by a nucleic-acid that hybridizes to the complement of SEQ ID NO: 1 under hybridization conditions comprising at least about 16% (v/v) formamide to at least about 30% (v/v) formamide and at least about 0.5M salt to at least about 0.9M salt at a temperature of 42°C.

109. **(Amended)** The method of claim **[12]**41, wherein the animal is a pig.

Please add the following claims:

114. (new) The vaccine composition of Claim 1 wherein said immunogenic component is selected from the group consisting of: SEQ ID NOS: 2, 4, 7, 9, 10, 12, 14, and 16.

115. (new) The vaccine composition of Claim 8, wherein said peptide or protein is encoded by the nucleotide sequence selected from the group consisting of: SEQ ID NOS: 1, 3, 5, 6, 8, 11, 13, 15, 17, 18, 19, 20, 21, 22, and 23.